AMITOSIS IN THE MALPIGHIAN TUBULES OF THE WALKING-STICK (DIAPHEROMERA FEMORATA).

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Schindler[1] in his paper on the Malpighian tubules of insects describes both direct cell division and budding of the nucleus. In amitotic division he notes a regular form in which the nucleus is first cut in two and then followed by the cell (in *Sarcophaga carnaria*), and also, in *Lophyrus pini*, a peculiar method in which the division passes from one side of the nucleus towards the other.

While working on the anatomy of the walking-stick it was often noticed, in studying sections through different parts of the body, that mitotic figures were quite abundant. In looking over serial sections cut through the head, thorax, or abdomen, mitosis was often observed in cells in the fat, tracheæ, epithelial cells of the ovarian tubules, etc., but nothing of the kind was ever seen in the Malpighian tubules. Later, all the slides in which any Malpighian tubules could be found, were reëxamined in an endeavor to find dividing nuclei. Other slides were then prepared, both sections and entire tubules, etc., and, while no mitotic figures could be found, some nuclei dividing amitotically were seen.

Attention has already been called[2] to the two kinds of Malpighian tubules in *Diapheromera femorata;* these differ in their size and in their position within the body of the insect. In both kinds of tubules the cells are binucleate.

The greater part of the material used was taken from mature or nearly full grown insects. Tubules were also prepared from a number of walking-sticks about one third full grown. Besides these two stages a number of the insects were hatched out in the laboratory and the tubules taken from some which were not more than four to six days old. Embryos in which the tubules had developed were also sectioned. This gave four different stages;

[1] E. Schindler, "Beiträge zur Kenntniss der Malpigi'schen Gefässe der Insecten," *Zeit. wiss. Zool.*, Vol. XXX., 1878.

[2] W. S. Marshall and H. H. Severin, "Ueber die Anatomie der Gespenstheuschrecke, *Diapheromera femorata*," *Arch. Biontol.*, Vol. I., 1906.

embryos, specimens but a few days old, insects one third full grown and mature insects.

In a longitudinal section through an embryo two or three weeks before it was ready to emerge from the egg, the Malpighian tubules were easily seen and the condition of the nuclei noted. The tubules were narrow and short but, proportional to the length of the body of the insect, were, I judge, about the same as in mature specimens. In these tubules a few mitotic figures were seen (Fig. 1) but no instance of a direct nuclear division could be found. It was impossible to find any cell boundaries and nothing could, at this stage, be determined as to the binucleate character of the cells. In the tubules of mature insects the nuclei often lie in pairs, the two of each pair being nearer to each other than to the others ; each pair being within a single cell. In the embryos studied the nuclei were very much crowded together and no such arrangement was possible ; this crowding together of the nuclei made the relationship in size of nucleus to cell very different from what was found in the mature insect where the cell was, in proportion to the size of its nuclei, very much greater than in the embryo.

In the tubules of very young walking-sticks, four to six days old, no dividing nuclei were seen. Both in structure and in relative size proportional to the tubule, or rather that part one might imagine to be the cell, the nuclei were here similar to those found in the embryo. Here and there two nuclei were seen with their opposing surfaces very close to or touching each other — if cell boundaries had been visible these two nuclei would, no doubt, have been within the same cell. In these young insects it was noticed that other organs did not show nearly so many mitotic figures as were found in maturer specimens.

In insects about one third grown the cells of the tubules were, in proportion to the nuclei, much larger than in the younger insects. In the old specimens, none of which had completed egg-laying and some had not yet begun, no mitotic figures were ever seen although, as already mentioned, nuclei dividing indirectly were fairly abundant in the other organs of the body. Thousands of cells were examined from the older insects and no trace of mitosis was ever seen in the Malpighian tubules.

There is no visible difference in the nuclear division in the large and in the small tubules, and, while nuclei from both kinds are figured, a description of one will hold good for both. The size of the nuclei of the larger tubules is much greater than of the smaller ones. Each nucleus contains a number of chromatin granules, held in a reticulum, and several nucleoles which stain with the safranin of Flemming's triple stain and with the fuchsin of an acid-fuchsin-methyl green solution. The nucleoles are of different sizes and are scattered irregularly within the nucleus: in division some pass to each of the daughter nuclei. The size of the nuclei in each tubule varies considerably as does their position, some lying with their long axis across and some with it parallel or oblique to the tubule. Most of the cells are arranged with their long axis parallel to the length of the tubule but they do not all occupy this position. The nuclei generally divided parallel with the tubule although some oblique ones were seen.

At the commencement of division the nucleus first assumes an irregularly oval outline very similar to the resting nuclei except a somewhat greater elongation (Fig. 5). The nucleus then narrows transversely (Fig. 2) until it becomes apparent that it is dividing amitotically (Figs. 3 and 4).[1] An examination of nuclei at this stage shows that the nucleoli are about to become fairly evenly distributed between the two daughter nuclei; all those not near the center of the nucleus continue in or near their original position while those near the center are pushed, as this part grows narrower, into one or the other of the daughter nuclei. As the central part becomes more of a connecting strand the two main portions change their outline becoming more circular and losing the elongated appearance they had in the earlier stage. Just after the completion of division the daughter nuclei project in a point towards each other showing where the connecting strand has severed (Fig. 6).

While most of the cells are binucleate it was found that a number of cells contained but a single nucleus. All cases of amitosis seen were found occurring in the nucleus of the uninucleate cells. In the binucleate cells amitosis was never seen.

[1] J. B. Carnoy, "La Cytodiérèse chez les Arthropodes." *La Cellule*, Vol. I., 1885.

It would seem that in the Malpighian tubules of the embryo some of the cells were left with but a single nucleus and that later, by amitosis, these cells became binucleate.

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EXPLANATION OF PLATE V.

All figures drawn with a camera-lucida.

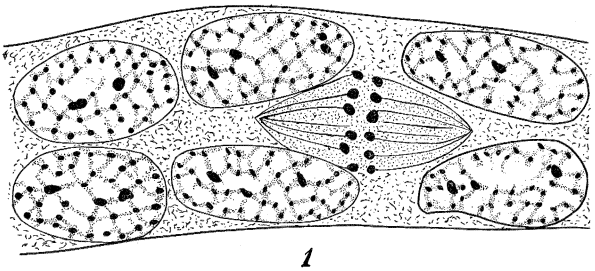
FIG. 1. Small piece of a Malpighian tubule from an embryo walking-stick. × 2,200.

FIG. 2. A nucleus showing early stage of amitotic division; from a large tubule. × 750.

FIGS. 3 and 4. Nuclei in a somewhat later stage; both from small tubules. × 1,200.

FIG. 5. Elongation of nucleus preparatory to division; from large tubule. × 1,200.

FIG. 6. Nucleus of large tubule after completed division. × 1,200.



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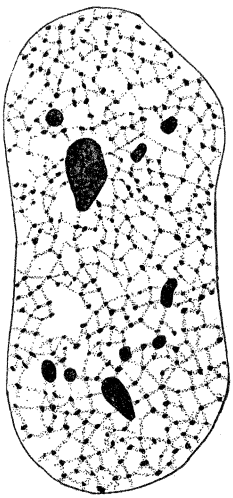

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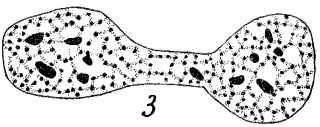

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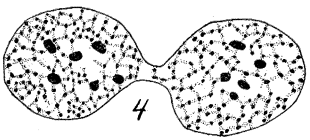

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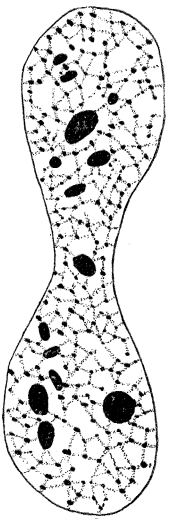

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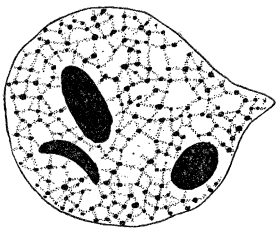

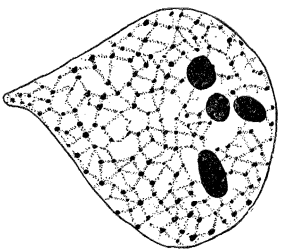

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